TECHNICAL REPORT

The Production of Methane and Nitrous Oxide Gas Standards for SCOR Working Group #143

John L. Bullister and David P. Wisegarver

National Oceanic and Atmospheric Administration

Pacific Marine Environmental Laboratory (NOAA-PMEL)

Samuel T. Wilson

Daniel K. Inouye Center for Microbial Oceanography: Research and Education (C-MORE)

Department of Oceanography, University of Hawaii

The production of Methane and Nitrous Oxide Gas Standards for Scientific Committee on Ocean Research (SCOR) Working Group #143

Report preface: The Scientific Committee on Oceanic Research (SCOR) is the leading international non-governmental organization for the promotion and coordination of international oceanographic activities, with the aim to solve conceptual and methodological problems that hinder marine research. The SCOR Working Group #143, formed in November 2013, and focuses on improving measurements of the nitrous oxide and methane in seawater. One activity conducted was the synthesis of gas standards which were distributed to the Full Members of the Working Group and a few Associate Members as listed below. This Technical Report provides details on the production of the nitrous oxide and methane standards and includes the absolute concentrations for each gas cylinder and best practice recommendations for gas regulator usage. Anyone seeking to cross-compare their own standards with these standards should contact one of the recipients of the standards.

Citation: Bullister, J. L., Wisegarver, D. P., & Wilson, S. T. (2016) The production of Methane and Nitrous Oxide Gas Standards for Scientific Committee on Ocean Research (SCOR) Working Group #143. pp 1-9.

Recipients of the gas standards
(Host institutions correct as of April 2016).

- Hermann Bange (GEOMAR - Helmholtz Centre for Ocean Research Kiel; Germany)
- John Bullister (National Oceanic and Atmospheric Administration Pacific Marine Environmental Laboratory; USA)
- Mercedes de la Paz Arándiga (Instituto de Investigaciones Marinas-CSIC; Spain)
- Laura Farias (Universidad de Concepción; Chile)
- Cliff Law (National Institute of Water and Atmospheric Research; New Zealand)
- Andy Rees (Plymouth Marine Laboratory; UK)
- Gregor Rehder (Institute for Baltic Sea Research in Warnemünde; Germany)
- Alyson Santoro (University of California, Santa Barbara; USA)
- Philippe Tortell (University of British Columbia; Canada)
- Rob Upstill-Goddard (University of Newcastle; UK)
- Sam Wilson (University of Hawaii; USA)
- Guiling Zhang (Ocean University of China; China)

BACKGROUND

Dissolved gas measurements of nitrous oxide and methane are conducted by disparate research laboratories across the globe as part of time-series measurements, hydrographic survey lines, and individual cruises. It is critical that these measurements are performed to a high level of accuracy and precision to determine the temporal and spatial variability which results from changes in atmospheric constituents, temperature, salinity, oxygen, nutrients and other influencing parameters. The Scientific Committee for Ocean Research (SCOR) Working Group #143 was formed to conduct and improve inter-laboratory comparisons for dissolved methane and nitrous oxide measurements. It quickly became apparent that while many laboratories have gas standards for calibrating methane and nitrous oxide concentrations in air and water samples, these standards were acquired from a variety of sources and were prepared using different techniques. In some cases, these standards have not been directly compared with standards at other laboratories. Most of these standards have methane and nitrous oxide concentrations similar to current atmospheric ratios, which can cause difficulty when using them to calibrate the very high methane and nitrous oxide concentrations found in some seawater samples. Funding was allocated for the preparation and distribution of two sets of high pressure gas standards- one with methane and nitrous oxide concentrations similar to modern air (air ratio standard-ARS) and the other set with higher methane and nitrous oxide concentrations for calibration of high concentration water samples (water ratio standard- WRS). These standards were prepared in the laboratory at NOAA-PMEL, checked for stability and assigned concentrations on the same calibration scale. One standard of each type was delivered to participating laboratories. These standards should provide a stable, common scale for the calibration and reporting of methane and nitrous oxide measurements made in the ocean over time scales of years to decades.

Pre-planning standard concentrations:

Two types of standards (Air Ratio Standards-ARS and Water Ratio Standards-WRS) were prepared by the Ocean Tracer Group at the NOAA Pacific Marine Environmental Laboratory (PMEL). Two sets of standards were prepared as the solubilities of methane and nitrous oxide in seawater are very different, with nitrous oxide about 23 times more soluble than methane in cold surface seawater (temperature ~-1.8^{o}C, salinity ~35). In some cases this makes it difficult to use ARS to directly calibrate methane and nitrous oxide extracted from small volume (~200 cc) discrete seawater samples. The standards were blended in Aculife 30Al high pressure aluminum cylinders (Scott Specialty Gas/Air Liquide) These cylinders have an internal volume of about 30 liters and were filled with standard at a total pressure of about 100 atm (1 atm = 1.01325 bar). The gas cylinders have an outlet connection that require a regulator fitted with a Compressed Gas Association (CGA) 580 fitting (USA & Canada).

ARS contained a blend of methane and nitrous oxide in pre-purified nitrogen (99.998%, NI 4.8). The amounts of methane, nitrous oxide and nitrogen in the ARS were chosen so that the concentration (mole fraction) ratios of methane/nitrogen and nitrous oxide/nitrogen in the ARS were in the range of the concentrations of these 2 trace gases in modern (2015) air. Modern air contains ~1800 nmol/mol (or ~1.8 ppm) methane, and ~340 nmol/mol (or ~340 ppb) nitrous oxide, where 1 nmol = 1 x 10^{-9} mol.

WRS also contained a blend of methane and nitrous oxide in pre-purified nitrogen (99.998%, NI 4.8). WRS were blended so that the amount of nitrous oxide and methane in ~3 cc of the WRS (at standard pressure of 1 bar and temperature of ~0°C) was approximately equal to the amount of dissolved methane and nitrous oxide in ~200 cc of cold surface seawater in equilibrium with the modern atmosphere. This can allow the calibration of seawater samples to be easier, since the amounts of methane and nitrous oxide in a small volume of WRS more closely match the amounts of these trace gases in the small volumes of seawater typically analyzed in discrete seawater samples.

Preparation and Analysis Techniques:

The ARS and WRS were prepared separately using similar techniques. 14 cylinders for ARS (or WRS) standards were connected to a high-pressure gas blending manifold.
The cylinder in position #1 (see Table 1 for ARS and Table 2 for WRS) was furthest from the high pressure inlet of the manifold and the cylinder in position #14 was closest to the inlet. The 14 cylinders were initially evacuated together on the manifold. A cylinder containing a mixture of nitrous oxide and methane in nitrogen at approximately 100 times the desired final concentrations ('spike' cylinder) was then attached to the manifold and this gas mixture was expanded into the 14 cylinders to a pressure slightly above 1 atm. The cylinders were then held for about 4 hours to allow the contents to come to equilibrium with the temperature in the laboratory. A vent valve on the manifold was then slowly opened so that the pressure in the manifold and cylinders came to a common equilibrium pressure equal to that of the ambient air. A high pressure (~150 atm) cylinder of pre-purified nitrogen was then connected to the manifold and nitrogen from this cylinder expanded individually into each cylinder in a stepwise fashion (starting from Cylinder #14 to Cylinder #1) until the cylinder reached a fixed target pressure. The first expansion increased the pressure of each standard cylinder from ~1 bar to ~15 bar. The pre-purified nitrogen cylinder was removed from the manifold and replaced with another ~150 bar cylinder. The expansion process was repeated with about 8 high pressure cylinders of nitrogen, and the final common pressures of the set of ARS (or WRS) cylinders reached ~100 bar.

Calibration of the Cylinders:

After preparation, to compare the relative nitrous oxide concentrations in the 14 cylinders, small fixed aliquots of gas (~3 cc) from each cylinder were analyzed on the PMEL CFC/SF6/N2O analytical system. To compare the relative methane concentration of each of the 14 cylinders, a small volume of gas from each cylinder was injected directly onto a chromatographic column and detected on an FID in a Shimadzu 8A gas chromatograph. The cylinders were run in random order on both systems.

One gas cylinder (cylinder PMEL426350 for ARS standards; cylinder PMEL464553 for WRS, see Tables 1 and 2, respectively) was chosen out of each group to act as a reference standard to compare with the methane and nitrous oxide concentrations in the other 13 cylinders of that group. The methane and nitrous oxide concentrations in PMEL426350 and PMEL464553 were initially assigned arbitrary values of 100 (see Tables 1 and 2). The comparisons for the ARS were conducted several times throughout an 18 month period to check for possible drift in the methane and nitrous oxide concentrations and over a 4 month period to check for drift in the WRS.

The range of relative concentrations for methane and nitrous oxide on the ARS standards was about 2%, with a standard deviation of less than 1% for both gases. (Tables 1 and 2). There was no significant drift in relative concentrations over an 18 month period. There appears to be a trend related to the position of the tank in the manifold during the blending and pressurization process, with the lowest concentration cylinders furthest from the nitrogen pressurizing entrance of the manifold. The lowest concentration cylinder PMEL416388 is about 1.8% lower than the highest tank PMEL416391, and in both gases, there appears to be an increase of about 1% between cylinders 7 and 8. These small difference among the relative concentrations in the 14 cylinders (typically <2%) shown in Tables 1 and 2 are possibly due to the order by which the 'spike gas' was introduced into the cylinders or to the order in which they were pressurized with nitrogen on the blending manifold. The results for the WRS group of standards were similar, with highest concentrations again in cylinders closest to the N2/spike entrance to the manifold. For the WRS, the range in relative concentrations was somewhat larger for nitrous oxide (stdev=2.8%) than methane (stdev=2.4%). (See Tables 1 and 2).

Nitrous Oxide Calibration:

PMEL standard cylinder PMEL72598 contains CFC-12, CFC-11, sulfur hexafluoride and nitrous oxide in nitrogen. The nitrous oxide concentration in this cylinder has been calibrated at the Scripps Institution of Oceanography (SIO) on the SIO 98 Calibration Scale, and assigned a nitrous oxide concentration of 324.07 nmol/mol. This cylinder was used to determine the concentration of nitrous oxide in ARS cylinder PMEL426350 (329.70 nmol/mol).

The remaining 13 ARS were assigned nitrous oxide concentrations by multiplying the nitrous oxide concentration in 426350 by the relative concentration of the cylinder to PMEL426350 (See Table 1).

In a similar fashion, the nitrous oxide concentration of WRS cylinder PMEL464553 was determined using the CFC/SF6/N2O analytical system, and comparing to cylinders PMEL72598, PMEL72602, PMEL72611 and PMEL_ARS_426350. The mean value determined for PMEL_WRS_646553 was 22746 nmol/mol. As was done above, this concentration was used to determine a nitrous oxide concentration in each of the WRS standards and reported in Table 2.

Methane calibration:

The relative concentrations of methane in the 14 ARS and 14 WRS measured at PMEL lab, based on measurements over a period of 16 months (ARS) and 4 months (WRS), appear to be stable within the analytical precision of the measurements and are in good agreement with the relative concentration ratios of nitrous oxide in the same set of cylinders.

A primary methane standard (CB10298), which consists of whole air collected and calibrated by the Global Monitoring Division (GMD) of NOAA in Boulder, CO. USA was assigned a methane value of 1965.324 nmol/mol (see Appendix A). Two of the standard cylinders (PMEL_ARS_416396 and PMEL_WRS_460867) were compared to the GMD standard by Dr. Sam Wilson at the University of Hawaii

(UH) to determine their absolute methane concentrations. Based on replicate analysis of ~0.5 ml and ~1 ml samples of these gases vs the GMD standard, concentrations of 1940 and 4540 nmol/mol were assigned to PMEL_ARS_416396 and PMEL_WRS_460867, respectively (see Table 1). In these comparisons, the response (peak areas) of the FID detector to methane concentration was assumed to be linear over the concentration range of the gases.
The methane concentration values for each of the remaining 13 ARS cylinders were determined by multiplying the assigned methane concentration of PMEL_ARS_416396 by the mean relative concentration ratio (Table 1) of each tank to PMEL_ARS_416396.

In a similar fashion, the methane concentration values for each of the remaining 13 WRS cylinders were determined by multiplying the assigned methane concentration of PMEL_WRS_460867 by the mean relative concentration ratio (Table 2) of each tank to PMEL_WRS_460867

There remains some uncertainty in the absolute methane calibrations for these cylinders. There were significant differences in the chromatographic peaks for methane generated by the ARS and WRS (where methane was blended in pre-purified nitrogen) and those from the GMD standard (methane in whole air). This may be due in part to incomplete chromatographic separation of CH_4 from oxygen and other components in the GMD whole air standards, as well as other factors. Users are strongly encouraged to compare their own standards with the ARS and ARS standards described here and report these comparisons to the authors of this report. Revisions of the nitrous oxide and methane concentrations assigned in Tables 1 and 2 may be made based on these comparisons and included in updates of this report.

Some suggestions for the care and use of these standards are provided in Appendix A.

Acknowledgements:
The Methane and Nitrous Oxide Gas Standards were produced via a Memorandum of Understanding between the University of Hawaii and NOAA-PMEL. Funding for the gas standards was provided by the U.S. National Science Foundation to the Scientific Committee on Ocean Research (OCE-1546580) and to the Center for Microbial Oceanography: Research and Education (EF0424599 to D Karl), the EU FP7 funded Integrated non-CO2 Greenhouse gas Observation System (InGOS) (Grant Agreement #284274), and NOAA’s Climate Program Office, Climate Observations Division. This is PMEL contribution #4603.

Table 1. SCOR Air Ratio Standards (ARS)

Methane Analysis:

Pos.	Cylinder#	Oct2014 RelCon	Jan2016 RelCon	Mean RelCon	Methane Conc. (nmol/mol)
1	PMEL416388	98.52		98.52	1924
2	PMEL416439	98.59	99.23	98.91	1932
3	PMEL426320	99.18	99.30	99.24	1938
4	PMEL426366	98.75	99.21	98.98	1933
5	PMEL426346	98.96	99.38	99.17	1937
6	PMEL416396	99.05	99.60	99.33	1940
7	PMEL426503	99.02	99.46	99.24	1938
8	PMEL426350	100.00	100.00	100.00	1953
9	PMEL426360	100.07	100.34	100.21	1957
10	PMEL426351	100.15	100.26	100.20	1957
11	PMEL416389	100.29	100.29	100.29	1959
12	PMEL416408	100.28	100.47	100.38	1961
13	PMEL426347	100.30	100.28	100.29	1959
14	PMEL416391	100.19		100.19	1957

Nitrous Oxide Analysis:

Pos.	Cylinder#	Aug2014 RelCon	Feb2015 RelCon	Jan2016 RelCon	Mean RelCon	N2O Conc. (nmol/mol)
1	PMEL416388	98.44	98.66		98.66	325.3
2	PMEL416439	98.55	99.04	98.68	98.86	325.9
3	PMEL426320	98.65	98.56	99.19	98.88	326.0
4	PMEL426366	98.38	99.11	98.92	99.02	326.5
5	PMEL426346	98.92	98.88	99.43	99.16	326.9
6	PMEL416396	99.05	98.92	100.46	99.69	328.7
7	PMEL426503	98.97	98.93	99.35	99.14	326.9
8	PMEL426350	100.00	100.00	100.00	100.00	329.7
9	PMEL426360	99.97	100.17	100.62	100.39	331.0
10	PMEL426351	100.42	100.45	100.61	100.53	331.5
11	PMEL416389	100.07	99.91	100.72	100.31	330.7
12	PMEL416408	100.39	100.19	101.04	100.61	331.7
13	PMEL426347	100.14	100.41	101.06	100.73	332.1
14	PMEL416391	99.94	100.40		100.40	331.0

RelCon= Relative Concentration
Methane Concentration of PMEL426350 = 1953 nmol/mol
Nitrous Oxide Concentration of PMEL426350 = 329.7 nmol/mol

Table 2. SCOR Water Ratio Standards (WRS)

Methane Analysis

Pos.	Cylinder#	Oct2015 RelCon	Jan2016 RelCon	Methane Conc. (nmol/mol)
1	PMEL460867	98.40	98.32	4540
2	PMEL464562	98.33	98.58	4552
3	PMEL464573	98.51	98.67	4556
4	PMEL460877	98.46	98.75	4560
5	PMEL464585	99.03	99.23	4582
6	PMEL460872	99.11	99.30	4585
7	PMEL464553	100.00	100.00	4618
8	PMEL460878	101.27	101.43	4684
9	PMEL464538	101.83	101.75	4698
10	PMEL464565	102.43	102.56	4736
11	PMEL464569	103.16	103.12	4762
12	PMEL464579	103.57	103.83	4794
13	PMEL464537	104.29	104.54	4827
14	PMEL460874	104.82	104.87	4843

Nitrous Oxide Analysis

Pos.	Cylinder#	Oct2015 RelCon	Jan2016 RelCon	N2O Conc. (nmol/mol)
1	PMEL460867	98.08	97.91	22271
2	PMEL464562	98.47	98.33	22367
3	PMEL464573	98.39	98.15	22326
4	PMEL460877	97.86	98.43	22389
5	PMEL464585	98.64	99.14	22549
6	PMEL460872	99.03	99.47	22627
7	PMEL464553	100.00	100.00	22746
8	PMEL460878	101.41	101.77	23148
9	PMEL464538	101.45	102.19	23244
10	PMEL464565	103.03	102.88	23401
11	PMEL464569	103.67	103.66	23578
12	PMEL464579	104.37	104.23	23708
13	PMEL464537	105.32	105.01	23885
14	PMEL460874	105.03	105.56	24012

RelCon= Relative Concentration
Methane Conc. of PMEL460867 = 4540 nmol/mol
Nitrous Oxide Conc of PMEL464553 = 22746

Appendix A: Pressure REGULATOR FLUSHING PROCEDURES:

The SCOR WG#143 methane/nitrous oxide standards are stored in specially treated Aculife aluminum high-pressure gas cylinders. The concentrations of methane and nitrous oxide in these calibrated standards are very low (ppm for methane; ppb for nitrous oxide).
Standard gas from these cylinders is typically delivered to an analytical system using a two-stage pressure regulator ('regulator') and a length of small diameter flexible copper or stainless steel tubing. Pressure gages are typically present on the first and second stages of the regulator. When the cylinder valve is opened, the first (high pressure) gage of the regular displays the cylinder pressure (typically ~100 atm.) and the second (low pressure) gage of the regulator displays the delivery pressure of the gas to the analytical system. A knob on the second-stage allows the delivery pressure of the gas to be adjusted to a desired value (typically less than 1 atm above ambient laboratory pressure). The actual flow rate to the analytical system is typically adjusted using a needle valve, and set to a measured value of 100 cc/minute or less.

Before attaching to the cylinder, a regulator is initially filled with laboratory air. This air must be thoroughly removed (flushed out) from the regulator to allow only pure standard gas to be transferred to the analytical system and to prevent laboratory air from back-diffusing from the regulator into the cylinder, which could contaminate the contents of the cylinder. This contamination could happen if a regulator is attached to a cylinder and the cylinder valve opened with no flow out of the regulator. To minimize back diffusion from the regulator into the cylinder and provide pure standard to the analytical system, the 2-stage pressure regulator should be flushed as follows:

1. Attach the regulator to the gas cylinder and tighten the CGA 590 fitting. If tubing leading from regulator vent to the analytical system is present, the tubing should be disconnected where it enters the sample loops of the analytical system. This will allow both the regulator and tubing to be flushed simultaneously.

2. Turn the regulator pressure knob (low pressure second-stage) clockwise enough turns so that when the first-stage of the regulator is pressurized, gas will immediately begin flowing out of the regulator and through the tubing.

3. Carefully open the cylinder valve a small amount to pressurize the first-stage, and immediately close the cylinder valve. Gas should flow through the regulator's second-stage and tubing to vent. As gas is escaping, adjust the second-stage pressure to a value slightly above ambient to allow flow to continue through the tubing.

4. When the first-stage (high pressure) gage approaches zero, quickly reopen the cylinder valve a small amount again and allow the first-stage of the regulator to re-pressurize, and then immediately close the cylinder valve, and allow gas to escape as in step 3 above.

5. Repeat steps 3 and 4 five times, so the regulator and tubing is flushed a total of six times.
During the last flush, as the first-stage pressure is approaching zero, open the cylinder valve fully, reconnect the tubing to the analytical system and

adjust the second-stage pressure slowly to the desired value (typically a slight amount above ambient). To assure consistency, the flow rates of the standard gas to the sample loops should be controlled using a in-line needle valve, measured with a flow meter and adjusted to a fixed value (ie. 50 cc/minute).

NOTES:
If a standard regulator had remained attached to a standard cylinder for a prolonged period with low (or no) flow, it is possible that low levels of contaminants (from components inside the regulator, etc.) can slowly accumulate inside. When starting to analyze standards after a break of several hours or longer, it is probably useful to bleed off gas held in regulator by disconnecting the outlet tubing at the CFC system, and flushing the regulator a few times as in steps 3-5 above.

SYSTEM STANDBY MODE:
When gas standards are not being run (and especially overnight or longer) the cylinder valve should be closed tightly and the second-stage regulator knob conpletely backed off (counterclockwise). This isolates the cylinder and keeps the regulator filled with standard, so that only 3 regulator flushes should be needed before a new set of samples are run.